AMENDMENTS TO THE SPECIFICATION

Please amend the specification at the paragraph bridging pages 1 and 2 as follows:

Mouse chromosome 2 has been linked to a variety of disorders including airway hyperresponsiveness and obesity (DeSanctis et al., *Nature Genetics*, 11: 150-154 (1995)); (Nagle *et al, Nature*, 398: 148-152 (1999)). This region of the mouse genome is homologous to portions of human chromosome 20 including 20p13-p12. Although human chromosome 20p13-12p has been linked to a variety of genetic disorders including diabetes insipidus, neurohypophyseal, congenital endothelial dystrophy of cornea, insomnia, neurodegeneration with brain iron accumulation 1 (Hallervorden-Spatz syndrome), fibrodysplasia ossificans progressiva, alagille syndrome, hydrometrocolpos (McKusick-Kaufman syndrome), Creutzfeldt-Jakob disease and Gerstmann-Straussler disease (See National Center for Biotechnology Information world wide web.ncbi.nlm.nih.gov/omim/); ~~http://www.ncbi.nlm.nih.gov/omim/~~), the genes affecting these disorders have yet to be discovered. There is a need in the art for identifying specific genes for such disorders because they are also associated with obesity, lung disease, particularly, inflammatory lung disease phenotypes such as Chronic Obstructive Lung Disease (COPD), Adult Respiratory Distress Syndrome (ARDS), and asthma. Identification and characterization of such genetic compositions will make possible the development of effective diagnostics and therapeutic means to treat lung related disorders.

Please amend the specification at page 7, lines 12-20 as follows:

To determine percent nucleotide or amino acid sequence similarity, sequences can be compared to publicly available sequence databases (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov; ~~www.ncbi.nlm.nih.gov~~) using the blastn2 algorithm (Altsch, *Nucl. Acids Res.*, 25:3389-3402 (1997)). The parameters for a typical search are: E=0.05, v=50, B=50 (where E is the expected probability score cutoff, V is the number of database entries returned in the reporting of the results, and B is the number of sequence alignments returned in the reporting of the results (Altsch et al, *J. Mol. Biol.*, 215:403-410 (1990)).

Please amend the specification at page 13, lines 11-26 as follows:

In a preferred embodiment, the protein or portion thereof has at least one function characteristic of a Gene 216 protein or polypeptide, for example, proteolysis, adhesion, fusion, and intracellular activity in the case of Gene 216 analogs, and/or antigenic function (*e.g.*, binding of antibodies that also bind to naturally occurring Gene 216 polypeptide). As such, these proteins are referred to as analogs, and include, for example, naturally occurring Gene 216, variants (*e.g.* mutants) of those proteins and/or

portions thereof. Such variants include mutants differing by the addition, deletion or substitution of one or more amino acid residues, or modified polypeptides in which one or more residues are modified, and mutants comprising one or more modified residues. The variant can have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, *e.g.*, replacement of leucine with isoleucine. More infrequently, a variant can have "nonconservative" changes, *e.g.*, replacement of a glycine with a tryptophan. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, for example, DNASTAR® software (DNASTAR®, Inc., Madison, Wis. 53715 U.S.A.).

Please amend the specification at page 33, line 24 through page 35, line 7 as follows:

Study subject genomic DNA (5 µl; 4.5 ng/µl) was amplified in a 10 µl PCR reaction using AmpliTaq Gold® DNA polymerase (0.225 U) and containing the final reaction components: 1X PCR buffer (80 mM $(NH_4)_2SO_4$, 30 mM Tris-HCl (pH 8.8), 0.5% Tween®-20 (polyoxyethylene (20) sorbitan monolaurate)), 200 µM each dATP, dCTP, dGTP and dTTP, 1.5-3.5 µM $MgCl_2$ and 250 µM forward and reverse PCR primers. PCR reactions were set up in 192 well plates (Costar) using a Tecan Genesis 150 robotic workstation equipped with a refrigerated deck. PCR reactions were overlaid with 20 µl mineral oil, and thermocycled on an MJ Research Tetrad DNA Engine equipped with four 192 well heads under the following conditions: 92° C for 3 min, 6 cycles of 92° C 30 sec, 56° C 1 min, 72° C 45 sec, followed by 20 cycles of 92° C 30 sec, 55° C 1 min, 72° C 45 sec and a 6 min incubation at 72° C. PCR products of 8-12 microsatellite markers were subsequently pooled using a Tecan Genesis 200 robotic workstation into two 96 well microtitre plates (2.0 µl PCR product from TET and FAM labeled markers, 3.01 HEX labeled markers) and brought to a final volume of 25 µl with H_2O. 1.9 µl of pooled PCR product was transferred to a loading plate and combined with 3.0 µl loading buffer (loading buffer is 2.5 l formamide/blue dextran (9.0 mg/ml), 0.5 µl GS-500 TAMRA labeled size standard, Perkin-Elmer/ABI division). Samples were denatured in the loading plate for 4 min at 95° C, placed on ice for 2 min, and electrophoresed in a 5% denaturing polyacrylamide gel (FMC on the ABI 377XL). Samples (0.8 µl) were loaded using an 8 channel Hamilton Syringe pipettor.

Each gel consisted of 62 study subjects and 2 control subjects (CEPH parents ID #1331-01 and 1331-02, Coriell Cell Repository, Camden, N.J.). Genotyping gels were scored in duplicate by investigators blind to patient identity and affection status using GENOTYPER® analysis software V 1.1.12 (ABI Division, Perkin Elmer Corporation). Nuclear families were loaded onto the gel with the parents flanking the siblings to facilitate error detection. Data with allele peak amplitude less than 100, as detected by GENESCAN® analysis software V 2.0.2 (ABI Division, Perkin Elmer Corporation), were either left unscored or rerun.

The final tables obtained from the Genotyper® output for each gel analysed were imported into a Sybase Database. Allele calling (binning) was performed using the SYBASE version of the ABAS® software (Ghosh et al, *Genome Research* 7:165-178 (1997)). Offsize bins were checked manually and incorrect calls were corrected or blanked. The binned alleles were then imported into the program MENDEL® (Lange et al., *Genetic Epidemiology*, 5, 471(1988)) for inheritance checking using the USERM13 subroutine (Boehnke et al, *AM. J. Hum. Genet.* 48:22-25 (1991)). Non-inheritance was investigated by examining the genotyping traces and once all discrepancies were resolved, the subroutine USERM13 was used to estimate allele frequencies.

Please amend the specification at page 35, line 22 through page 36, line 19 as follows:

The entire human genome is 3,300 cM long. In order to find an unknown disease gene within 5-10 cM of a marker locus, the whole human genome can be searched with roughly 330 informative marker loci spaced at approximately 10 cM intervals (Botstein et al, Am. J. Hum. Genet., 32:314-331 (1980)). The reliability of linkage results is established by using a number of statistical methods. The methods most commonly used for the detection by linkage analysis of oligogenes involved in the etiology of a complex trait are non-parametric or model-free methods which have been implemented into the computer programs MAPMAKER/SIBS (Kruglyak L & Lander E S, *Am J Hum Genet* 57:439-454, 1995) and GENEHUNTER® (Kruglyak L et al., Am J Hum Genet 58:1347-1363, 1996). Linkage analysis is performed by typing members of families with multiple affected individuals at a given marker locus and evaluating if the affected members (excluding parent-offspring pairs) share alleles at the marker locus that are identical by descent (IBD) more often than expected by chance alone. As a result of the rapid advances in mapping the human genome over the last few years, and concomitant improvements in computer methodology, it has become feasible to carry out linkage analyses using multi-point data. Multi-point analysis provides a simultaneous analysis of linkage between the trait and several linked genetic markers, when the recombination distance among the markers is known. A LOD score statistic is computed at multiple locations along a chromosome to measure the evidence that a susceptibility locus is located nearby. A LOD score is the logarithm base 10 of the ratio of the likelihood that a susceptibility locus exists at a given location to the likelihood that no susceptibility locus is located there. By convention, when testing a single marker, a total LOD score greater than +3.0 (that is, odds of linkage being 1,000 times greater than odds of no linkage) is considered to be significant evidence for linkage.

Please amend the specification at page 37, lines 11-18 as follows:

Allele sharing methods, implemented in the MAPMAKER/SIBS (Kruglyak L & Lander E S, *Am J Hum Genet* 57:439-454, 1995), were used on our sample of 462 nuclear with affected sibling pairs. Multipoint linkage analyses were performed using 23 polymorphic markers spanning a 95 cM region on both arms of chromosome 20. The

map location and distances between markers were obtained from the genetic maps published by the Marshfield medical research foundation (world wide web.marshmed.org/genetics/)(~~http://www.marshmed.org/genetics/~~). Ambiguous order in the Marshfield map was resolved using the program MULTIMAP (Matise T C et al., *Nature Genet* 6:384-390, 1994).

Please amend the specification at page 41, lines 6-26 as follows:

1. Map Integration. Various publicly available mapping resources were utilized to identify existing STS markers (Olson et al, (1989), *Science*, 245:1434-1435) in the 20p13-p12 region. Resources included the Genome Database (GDB; world wide web: gdbwww.gdb.org/ ~~http://gdbwww.gdb.org/~~), Genethon (world wide web.genethon.fr/genethon_en.html) (~~http://www.genethon.fr/genethon_en.html~~), Marshfield Center for Medical Genetics (world wide web.marshmed.org/genetics/) (~~http://www.marshmed.org/genetics/~~), the Whitehead Institute Genome Center (world wide web.genome.wi.mit.edu/) (~~http://www-genome.wi.mit.edu/~~), GeneMap98, dbSTS and dbEST (NCBI, world wide web.ncbi.nlm.nih.gov/) ~~http://www.ncbi.nlm.nih.gov/~~), the Sanger Centre (world wide web.sanger.ac.uk/) (~~http://www.sanger.ac.uk/~~), and the Stanford Human Genome Center (world wide web.shgc.stanford.edu/) (~~http://www.shgc.stanford.edu/~~). Maps were integrated manually to identify markers mapping to the disorder region. A list of the markers is provided in Table 1.

2. Marker Development. Sequences for existing STSs were obtained from the GDB, RHDB (world wide web.ebi.ac.uk/RHdb/) (~~http://www.ebi.ac.uk/RHdb/~~), or NCBI and were used to pick primer pairs (overgos, See Table 2) for BAC library screening. Novel markers were developed either from publicly available genomic sequences, proprietary cDNA sequences or from sequences derived from BAC insert ends (described below). Primers were chosen using a script that automatically performs vector and repetitive sequence masking using Crossmatch (P. Green, U. of Washington); subsequent primer picking was performed using a customized Filemaker® Pro database. Primers for use in PCR-based clone confirmation or radiation hybrid mapping (described below) were chosen using the program Primer3 (Steve Rozen, Helen J. Skaletsky (1996, 1997); Primer3 is available at world wide web.genome.wi.mit.edu/genome_software/othe- r/primer3.html) ~~http://www-genome.wi.mit.edu/genome_software/othe-r/primer3.html~~).

Please amend the specification at page 42, line 7 – page 43, line 13 as follows:

3. Radiation Hybrid (RH) Mapping. Radiation hybrid mapping was performed against the Genebridge4 panel (Gyapay, et al., (1996), *Hum. Mol. Genet.* 5:339-46) purchased from Research Genetics, in order to refine the chromosomal localization of genetic markers used in genotyping as well as to identify, confirm and refine localizations of markers from proprietary sequences. Standard PCR procedures were

used for typing the RH panel with markers of interest. Briefly, 10 µl PCR reactions contained 25 ng DNA of each of the 93 Genebridge4 RH samples. PCR products were electrophoresed in 2% agarose gels (Sigma) containing 0.5 µg/ml ethidium bromide in 1X TBE at 150 volts for 45 min. The electrophoresis units used were the Model A3-1 systems from Owl Scientific Products. Typically, gels contained 10 tiers of lanes with 50 wells/tier. Molecular weight markers (100 bp ladder, GIBCO/BRL) were loaded at both ends of the gel. Images of the gels were captured with a Kodak DC40 CCD camera and processed with Kodak 1D software. The gel data were exported as tab delimited text files; names of the files included information about the panel screened, the gel image files and the marker screened. These data were automatically imported using a customized Perl script into Filemaker® databases for data storage and analysis. The data were then automatically formatted and submitted to an internal server for linkage analysis to create a radiation hybrid map using RHMAPPER (Stein, L., Kruglyak, L., Slonim, D., and El Lander (1995); available from the Whitehead Institute/MIT Center for Genome Research, at world wide web.genome.wi.mit.edu/ftp/pub/software/rhmapper/ http://www.genome.wi.mit.edu/ftp/pub/software/rhmapper/, and via anonymous ftp to filed transfer protocol.genome.wi.mit.edu ftp.genome.wi.mit.edu, in the directory /pub/software/rhmapper.)

4. BAC Library Screening. The protocol used for BAC library screening was based on the "overgo" method, originally developed by John McPherson at Washington University in St. Louis (world wide web.tree.caltech.edu/protocols/overgo.html (http://www.tree.caltech. edu/protocols/overgo.html, and Cai, W-W., et al., (1998), *Genomics* 54:387-397). This method involves filling in the overhangs generated after annealing two primers, each 22 nucleotides in length, that overlap by 8 nucleotides. The resulting labeled 36 bp product is then used in hybridization-based screening of high density grids derived from the RPCI-11 BAC library (Pieter deJong, Roswell Park Cancer Institute, world wide web: bacpac.med.buffalo.edu http://bacpac.med.buffalo.edu). Typically, 15 probes were pooled together in one hybridization of 12 filters (13.5 genome equivalents).

Stock solutions (2 µM) of combined complementary oligos were heated at 80° C for 5 min. then placed at 37° C for 10 min followed by storage on ice. Labeling reactions were set up as follows: 1.0 µl H_2O, 5 µl mixed oligo--2 µM each, 0.5 µl BSA (2 mg/ml), 2 µl OLB(-A, -C, -N6) Solution (see below), 0.5 µl .sup.32P-dATP (3000 Ci/mmol), 0.5 µl .sup.32P-dCTP (3000 Ci/mmol), 0.5 µl Klenow fragment (5U/µl). The reaction was incubated at room temperature for 1 hr followed by removal of unincorporated nucleotides with Sephadex® G50 spin columns (*i.e.*, cross-linked dextran beads).

Please amend the specification at page 43, line 22 through page 45, line 5 as follows:

High density BAC library membranes were pre-wetted in 2X SSC at 58° C. Filters were then drained slightly and placed in hybridization solution (1% Bovine serum

albumin, 1 mM EDTA-pH 8.0, 7% SDS, and 0.5 M sodium phosphate) pre-warmed to 58° C and incubated at 58° C for 2-4 hr. Typically, 6 filters were hybridized per container. Ten ml of pre-hybridization solution were removed, combined with the denatured overgo probes, and added back to the filters. Hybridization was performed overnight at 58° C. The hybridization solution was removed and filters were washed once in 2X SSC, 0.1% SDS, followed by a 30 minute wash in the same solution but at 58° C. Filters were then washed in 1.5X SSC, 01.% SDS at 58° C for 30 min. 0.5X SSC, 0.1% SDS at 58° C for 30 min and finally in 0.1X SSC, 0.1% SDS at 58° C for 30 min. Filters were then wrapped in Saran® Wrap *(i.e., plastic wrap)* and exposed to film overnight. To remove bound probe, filters were treated in 0.1X SSC, 0.1% SDS pre-warmed to 95° C and allowed to return to room temperature. Clone addresses were determined as described by instructions supplied by RPCI.

Recovery of clonal BAC cultures from the library involved streaking out a sample from the appropriate library well onto LB agar (Maniatis, T., Fritsch, E. F., and J. Sambrook, (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) containing 12.5 µg/ml chloramphenicol (Sigma) and incubating overnight. A single colony and a portion of the initial streak quadrant were inoculated into 400 µl LB plus chloramphenicol in wells of a 96 well plate. Cultures were grown overnight at 37° C For storage, 100 µl of 80% glycerol was added and the plates placed at -80° C To determine the marker content of clones, aliquots of the 96 well plate cultures were transferred to the surface of nylon filters (GeneScreen Plus®, NEN) placed on LB/chloramphenicol Petri plates. Colonies were grown overnight at 37° C and colony lysis was performed as follows: Filters were placed on pools of 10% SDS for 3 min, 0.5 N NaOH, 1.5 M NaCl for 5 min, and 0.5 M Tris-HCl, pH 7.5, 1 M NaCl for 5 min. Filters were then air dried and washed free of debris in 2xSSC for 1 hr. The filters were air dried for at least 1 hr and DNA crosslinked linked to the membrane using standard conditions. Probe hybridization and filter washing were performed as described above for the primary library screening. Confirmed clones were stored in LB containing 15% glycerol.

In some cases polymerase chain reaction (PCR) was used to confirm the marker content of clones. PCR conditions for each primer pair were initially optimized with respect to $MgCl_2$ concentration. The standard buffer was 10 mM Tris-HCl (pH 8.3), 50 mM KCl, $MgCl_2$, 0.2 mM each dNTP, 0.2 µM each primer, 2.7 ng/µl human DNA, 0.25 units of AmpliTaq® (Perkin Elmer) and $MgCl_2$ concentrations of 1.0 mM, 1.5 mM, 2.0 mM or 2.4 mM. Cycling conditions included an initial denaturation at 94° C for 2 minutes followed by 40 cycles at 94°C for 15 seconds, 55°C for 25 seconds, and 72°C for 25 seconds followed by a final extension at 72°C for 3 minutes. Depending on the results from the initial round of optimization the conditions were further optimized if necessary. Variables included increasing the annealing temperature to 58°C or 60°C, increasing the cycle number to 42 and the annealing and extension times to 30 seconds, and using AmpliTaqGold® (Perkin Elmer).

Please amend the specification at page 47, lines 10-17 as follows:

NotI digests were analyzed on a ~~CHEF-DRII~~ CHEF-DR®II (BioRad) electrophoresis unit according to the manufacturer's recommendations. Briefly, 1% agarose gels (BioRad pulsed field grade) were prepared in 0.5X TBE, equilibrated for 30 min in the electrophoresis unit at 14° C, and electrophoresed at 6 volts/cm for 14 hrs with circulation. Switching times were ramped from 10 sec to 20 sec. Gels were stained after electrophoresis in 0.5 µg/ml ethidium bromide. Molecular weight markers included undigested lambda DNA, *Hind*III digested lambda DNA, lambda ladder PFG ladder, and low range PFG marker (all from New England Biolabs).

Please amend the specification at page 47, lines 23-30 as follows:

pBAC 5'-2 TGT AGG ACT ATA TTG CTC SEQ ID NO.:30

pBAC 3'-1 CGA CAT TTA GGT GAC ACT SEQ ID NO.:31

The following sequencing protocol using ABI dye-terminator chemistry was used to set up sequencing reactions for 96 clones. The BigDye® (Mix: Perkin Elmer/ABI BigDye®) Terminator Ready Reaction Mix with AmpliTaq®" FS, Part number 4303151, was used for sequencing with fluorescently labelled dideoxy nucleotides. A master sequencing mix was prepared for each primer reaction set including:

1600 µl of BigDye® terminator mix (ABI)

Please amend the specification at page 48, lines 17-23 as follows:

At the end of the sequencing reaction, the plates were removed from the thermocycler and centrifuged briefly. Centri-Sep™ 96 plates were then used according to manufacturer's recommendation to remove unincorporated nucleotides, salts and excess primers. Each sample was resuspended in 1.5 µl of loading dye of which 1.3 µl was loaded on ABI 377 Fluorescent Sequencers. The resulting endsequences were then used to develop markers to rescreen the BAC library for filling gaps and were also analyzed by BLAST searching for EST or gene content.

Please amend the specification at page 49, line 23 through page 50, line 11 as follows:

The purified DNA fragments were then blunt-ended using T4 DNA polymerase. The healed DNA was then ligated to unique *Bst*XI-linker adapters (5' GTCTTCACCACGGGG; SEQ ID NO.:32 and 5' GTGGTGAAGAC; SEQ ID NO.:33 in 100-1000 fold molar excess). These linkers are complimentary to the BstXI-cut pMPX

vectors, while the overhang is not self-complimentary. Therefore, the linkers will not concatemerize nor will the cut-vector re-ligate to itself easily. The linker-adapted inserts were separated from unincorporated linkers on a 1% agarose gel and purified using GeneClean® (BIO 101, Inc.). The linker-adapted insert was then ligated to a modified pBlueScript vector to construct a "shotgun" subclone library. The vector contains an out-of-frame lacZ gene at the cloning site which becomes in-frame in the event that an adapter-dimer is cloned, allowing these to be avoided by their blue color.

All subsequent steps were based on sequencing by ABI377 automated DNA sequencing methods. Only major modifications to the protocols are highlighted. Briefly, the library was then transformed into DH5™-competent cells (Gibco/BRL, DH5™-transformation protocol). Quality was assessed by plating onto antibiotic plates containing ampicillin and IPTG/Xgal. The plates were incubated overnight at 37° C. Successful transformants were then used for plating of clones and picking for sequencing. The cultures were grown overnight at 37°C. DNA was purified using a silica bead DNA preparation (Ng et al, *Nucl. Acids Res.*, 24:5045-5047 (1996)) method. In this manner, 25 µg of DNA was obtained per clone.

Please amend the specification at page 50, lines 21-30 as follows:

Any gene or EST mapping to the interval based on public map data or proprietary map data was considered a candidate respiratory disease gene. Public map data were derived from several sources: the Genome Database (GDB, world wide web: gdbwww.gdb.org/ ~~http://gdbwww.gdb.org/~~), the Whitehead Institute Genome Center (world wide web-genome.wi.mit.edu/) (~~http://www-genome.wi.mit.edu/~~), GeneMap98, UniGene, OMIM, dbSTS and dbEST (NCBI, world wide web.ncbi.nlm.nih.gov/ ~~http://www.ncbi.nlm.nih.gov/~~), the Sanger Centre (world wide web.snager.ac.uk/) (~~http://www.sanger.ac.uk/~~), and the Stanford Human Genome Center (world wide web.shgc.stanford.edu/) (~~http://www-shgc.stanford.edu/~~). Proprietary data was obtained from sequencing genomic DNA (cloned into BACs) or cDNAs (identified by direct selection, screening of cDNA libraries or full length sequencing of IMAGE Consortium (world wide web-bio.11nl.gov/bbrp/image.html) (~~http://www-bio.11nl.gov/bbrp/image.html~~) cDNA clones).

Please amend the specification at page 50, line 31 through page 51, line 7 as follows:

1. Gene Identification from clustered DNA fragments. DNA sequences corresponding to gene fragments in public databases (Genbank and human dbEST) and proprietary cDNA sequences (IMAGE consortium and direct selected cDNAs) were masked for repetitive sequences and clustered using the PANGEA Systems® (Oakland, Calif.) EST clustering tool. The clustered sequences were then subjected to computational analysis to identify regions bearing similarity to known genes. This

protocol included the following steps:

Please amend the specification at page 51, lines 8-30 as follows:

i. The clustered sequences were compared to the publicly available Unigene database (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.hih.gov www.ncbi.nlm.nih.gov) using the blastn2 algorithm (Altschul et al, *Nucl. Acids Res.*, 25:3389-3402 (1997)). The parameters for this search were: E=0.05, v=50, B=50 (where E is the expected probability score cutoff, V is the number of database entries returned in the reporting of the results, and B is the number of sequence alignments returned in the reporting of the results (Altschul et al, *J. Mol. Biol.*, 215:403-410 (1990)).

ii. The clustered sequences were compared to the Genbank database (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov www.ncbi.nlm.nih.gov) using blastn2 (Altschul et al, *Nucl. Acids. Res.*, 25:3389-3402 (1997)). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B are defined as above.

iii. The clustered sequences were translated into protein for all six reading frames, and the protein sequences were compared to a non-redundant protein database compiled from Genpept Swissprot PIR (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov www.ncbi.nlm.nih.gov). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B are defined as above.

Please amend the specification at page 52, lines 11 - 17 as follows:

ii. BAC vector sequences were "masked" within the sequence by using the program crossmatch (Phil Green, world wide web: chimera.biotech.washington.edu.backslash.UWGC http://chimera.biotech.washington.edu.backslash.UWGC). Since the shotgun library construction detailed above left some BAC vector in the shotgun libraries, this program was used to compare the sequence of the BAC contigs to the BAC vector and to mask any vector sequence prior to subsequent steps. Masked sequence was marked by an "X" in the sequence files, and remained inert during subsequent analyses.

Please amend the specification at page 52, lines 28 - page 53 line 13 as follows:

vi. The sequence was compared to the publicly available unigene database (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov ~~www.ncbi.nlm.nih.gov~~) using the blastn2 algorithm (Altschul et al, *Nucl. Acids Res.*, 25:3389-3402 (1997)). The parameters for this search were: E=0.05, v=50, B=50 (where E is the expected probability score cutoff, V is the number of database entries returned in the reporting of the results, and B is the number of sequence alignments returned in the reporting of the results (Altschul et al, *J. Mol. Biol.*, 215:403-410 (1990)).

vii. The sequence was translated into protein for all six reading frames, and the protein sequences were compared to a non-redundant protein database compiled from Genpept Swissprot PIR (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov ~~www.ncbi.nlm.nih.gov~~). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B are defined as above.

viii. The BAC DNA sequence was compared to a database of clustered sequences using blastn2 (Altschul et al, *Nucl. Acids. Res.*, 25:3389-3402 (1997)). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B are defined as above. The database of clustered sequences was prepared utilizing a proprietary clustering technology (Pangea Systems®, Inc.) using cDNA clones derived from direct selection experiments (described below), human dbEST mapping to the 20p13-p12 region, proprietary cDNAs, Genbank genes and IMAGE consortium cDNA clones.

Please amend the specification at page 53, line 25 - page 54 line 11 as follows:

x. The BAC sequence was compared to the Genbank database (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov ~~www.ncbi.nlm.nih.gov~~) using blastn2 (Altschul et al, *Nucl. Acids. Res.*, 25:3389-3402 (1997)). The parameters for this search were E=0.05, V=50, B=50; where E, V, and B are defined as above.

xi. The BAC sequence was compared to the STS division of Genbank database (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov ~~www.ncbi.nlm.nih.gov~~) using blastn2 (Altschul et al., 1997). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B are defined as above.

xii. The BAC sequence was compared to the Expressed Sequence Tag (EST) Genbank database (National Center for Biotechnology Information, National Library of Medicine, 38A, 8N905, 8600 Rockville Pike, Bethesda, Md. 20894; world wide web.ncbi.nlm.nih.gov www.ncbi.nlm.nih.gov) using blastn2 (Altschul et al., *Nucl. Acids. Res.*, 25:3389-3402 (1997)). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B are defined as above.

Please amend the specification at page 55, lines 5 through page 56, line 14 as follows:

Total RNA was isolated from normal and asthmatic lung tissue using TRIzol® Reagents (Gibco BRL, Rockville, Md.) which are ready-to-use monophasic solutions of guanadinium isothiocyanate and phenol (Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem., 162:156-159; Chomczynski, P., Bowers-Finn, R., and Sabatini, L. (1987) J. NIH Res. 6:83; Simms, D., Cizdiel, P. E., and Chomczynski, P. (1993) Focus 15:99; Chomczynski, P. (1993) BioTechniques 15:532). Five hundred milligrams of frozen tissue was crushed into a fine powder using a Bessman tissue pulverizer (Fisher Scientific). The TRIzol® Reagents were mixed with the crushed tissue according to the manufacturer's recommendations to isolate total RNA.

To ascertain whether there was genomic DNA or heteronuclear RNA contamination within the RNA isolates, PCR and RT/PCR were performed, respectively. The PCR analysis was performed using primers (Research Genetics) that amplify STS markers from chromosomes 2 (D2S2358), 7 (D7S2776, D7S685), 10 (D10S228, D10S1755) and 20 (D20S905, D20S95). All PCR reactions were performed in a volume of 25 µl that contained 1 µl of RNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 200 mM each dNTPs, 10 µM of each primer and 1 unit Taq DNA polymerase (Perkin Elmer). A Perkin Elmer 9600 was used to amplify the material under the following conditions: 30 seconds at 94° C, 30 seconds at 55° C and 30 seconds at 72° C for 30 cycles. The RT/PCR analysis was performed using the SuperScript™ One-Step RT-PCR System (Gibco-BRL, Rockville, Md.) according to the manufacturer's recommendations. All PCR and RT/PCR products were evaluated by electrophoresis on a 1% agarose gel.

Poly A+ RNA was prepared from the total RNA isolated from the human primary cells and lung tissues using Dynabeads® Oligo(dT) (*i.e.*, magnetic separation beads) according to the manufacturer's recommendations (Dynal, Lake Success, N.Y.). Approximately 4 µg of messenger RNA was isolated from 150 µg of total RNA for each cell type and tissue source. Total RNA isolated from brain tissue was purchased from Clontech (Palo Alto, Calif.) and poly A+ RNA was prepared from this material using the Dynabeads® Oligo(dT) as described above.

Oligo dT and random primed cDNA pools were generated from the mRNA isolated from each cell type and tissue source. Briefly, 2.0 µg mRNA was mixed with

oligo(dT) primer in one reaction, and 2.0 µg mRNA was mixed with random hexamers in another reaction, and converted to double stranded complementary DNA using the SuperScript™ Choice System for cDNA Synthesis (Gibco-BRL, Rockville, Md.) according to manufacturer's recommendations.

Please amend the specification at page 59, lines 10-17 as follows:

The amplified material was cloned into the UDG vector pAMP10 (Gibco-BRL, Rockville, Md.) in accordance with the manufacturer's recommendations. Four hundred and eighty clones were picked from each transformed source and arrayed into five 96 well microtiter plate. Each selected cDNA library was stamped, in duplicate, in a high density format onto Hybond N+ nylon membrane™ (Amersham). The bacteria were grown overnight at 37° C, and the membranes were processed as recommended by the manufacturer.

Please amend the specification at page 60, lines 5-28 as follows:

Further background clones such as high copy repeats, ribosomal RNA, plasmid, mitochondrial, *E. coli* and yeast that were not identified in the hybridization process were removed from the dataset using in silico methods. This process yielded 787 cDNA clones for further analysis. These clones were clustered using Pangea System®'s EST Clustering Tool (Oakland, Calif.) and analyzed with BLASTN, X and FASTA programs. This software tool enables one to construct full length gene sequences by aligning the DNA fragments.

These direct selected clones were combined with the proprietary cDNA sequences, and sequences within the public domain (dbEST and Genbank) then clustered using the Pangea System®s EST Clustering Tool. These clustered sequences are known to those skilled in the art as consensus sequences assisted in extending the gene sequences disclosed herein.

c. Mapping Analysis.

Those BACs that were identified, and mapped to the region 20p13-p12 were used to determine which cDNA clones map back using standard hybridization methods as described by Sambrook et al, (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y. The DNA from each BAC was isolated using Nucleobond® AX columns as described by the manufacturer (Clontech, Palo Alto, Calif.) and hybridized at 65° C to high density filters containing the sequenced cDNAs. Those cDNAs that showed duplicate signals were scored as mapping back to the genomic clone and to the region. These cDNAs were studied further as disorder associated gene(s).

Please amend the specification at page 61, line 7 through page 63, line 7 as follows (Please note that "Construction of cDNA libraries" is not being added, rather it is underlined in the specification as filed):

1. Construction of cDNA libraries. Directionally cloned cDNA libraries from normal lung and bronchial epithelium were constructed using standard methods described previously (Soares et. al., 1994, Automated DNA Sequencing and Analysis, Adams, Fields and Venter, Eds., Academic Press, NY, pages 110-114). Total and cytoplasmic RNAs were extracted from tissue or cells by homogenizing the sample in the presence of Guanidinium Thiocyanate-Phenol-Chloroform extraction buffer (e.g. Chomczynski and Sacchi, Anal. Biochem., 162:156-159 (1987)) using a polytron homogenizer (Brinkman Instruments). PolyA+ RNA was isolated from total/cytoplasmic RNA using dynabeads®-dT (*i.e.*, magnetic separation beads) according to the manufacturer's recommendations (Dynal, Inc.). The ds cDNA synthesized was then ligated into the plasmid vector pBluescript II KS+ (Stratagene, La Jolla, Calif.), and the ligation mixture was transformed into E. coli host DH10B or DH12S by electroporation (Soares, 1994). Following overnight growth at 37° C, DNA was recovered from the E. coli colonies after scraping the plates by processing as directed for the Mega-prep® kit (Qiagen, Chatsworth, Calif.). The quality of the cDNA libraries was estimated by counting a portion of the total number of primary transformants, determining the average insert size and the percentage of plasmids with no cDNA insert. Additional cDNA libraries (human total brain, heart, kidney, leukocyte, and fetal brain) were purchased from Life Technologies, Bethesda, Md.

cDNA libraries, both oligo (dT) and random hexamer-primed were used for isolating cDNA clones mapping within the disorder critical region. Four 10 x10 arrays of each of the cDNA libraries were prepared as follows: the cDNA libraries were titered to 2.5 x 10^6 using primary transformants. The appropriate volume of frozen stock was used to inoculate 2 L of LB/ampicillin (100 µg/µl). 400 aliquots containing 4 ml of the inoculated liquid culture were generated. Each tube contained about 5000 cfu. The tubes were incubated at 30° C overnight with shaking until an OD of 0.7-0.9 was obtained. Frozen stocks were prepared for each of the cultures by aliquotting 300 µl of culture and 100 µl of 80% glycerol. Stocks were frozen in a dry ice/ethanol bath and stored at -70° C. DNA was isolated from the remaining culture using the Qiagen (Chatsworth, Calif.) ~~Spin mini-prep~~ QIAprep Spin Mini-Prep® it according to the manufacturer's instructions. The DNAs from the 400 cultures were pooled to make 80 column and row pools. Markers were designed to amplify putative exons from candidate genes. Once a standard PCR condition was identified and specific cDNA libraries were determined to contain cDNA clones of interest, the markers were used to screen the arrayed library. Positive addresses indicating the presence of cDNA clones were confirmed by a second PCR using the same markers.

Once a cDNA library was identified as likely to contain cDNA clones corresponding to a specific transcript of interest from the disorder critical region, it was

used to isolate a clone or clones containing cDNA inserts. This was accomplished by a modification of the standard "colony screening" method (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y. (1989)). Specifically, twenty 150 mm LB+ampicillin agar plates were spread with 20,000 colony forming units (cfu) of cDNA library and the colonies allowed to grow overnight at 37° C. Colonies were transferred to nylon filters (Hybond™ from Amersham, or equivalent) and duplicates prepared by pressing two filters together essentially as described (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y. (1989)). The "master" plate was then incubated an additional 6-8 hrs to allow the colonies additional growth. The DNA from the bacterial colonies was then bound onto the nylon filters by treating the filters sequentially with denaturing solution (0.5 N NaOH, 1.5 M NaCl) for two minutes, neutralization solution (0.5 M Tris-Cl pH 8.0, 1.5 M NaCl) for two minutes (twice). The bacterial colonies were removed from the filters by washing in a solution of 2X SSC/2% SDS for one minute while rubbing with tissue paper. The filters were air dried and baked under vacuum at 80° C for 1-2 hrs to cross link the DNA to the filters.

Please amend the specification at page 64, line 7 through page 65, line 25 as follows (Please note that "Expression Analysis" and "RT-PCR" are not being added, rather they are underlined in the specification as filed):

Rapid Amplification of cDNA ends (RACE) was performed following the manufacturer's instructions using a Marathon™ cDNA Amplification Kit (Clontech, Palo Alto, Calif.) as a method for cloning the 5' and 3' ends of candidate genes. cDNA pools were prepared from total RNA by performing first strand synthesis, where a sample of total RNA sample was mixed with a modified oligo (dT) primer, heated to 70° C, cooled on ice and followed by the addition of: 5X first strand buffer, 10 mM dNTP mix, and AMV Reverse Transcriptase (20 U/µl). The reaction mixture was incubated at 42° C for an hour and placed on ice. For second strand synthesis, the following components were added directly to the reaction tube: 5X second strand buffer, 10 mM DNTP mix, sterile water, 20X second strand enzyme cocktail and the reaction tube was incubated at 16° C for 1.5 hours. T4 DNA Polymerase was added to the reaction tube and incubated at 16° C for 45 minutes. The second-strand synthesis was terminated with the addition of an EDTA/Glycogen mix. The sample was subjected to a phenol/chloroform extraction and an ammonium acetate precipitation. The cDNA pools were checked for quality by analyzing on an agarose gel for size distribution. Marathon™ cDNA adapters were then ligated onto the cDNA ends. The specific adapters contained priming sites that allowed for amplification of either 5' or 3' ends, and varied depending on the orientation of the gene specific primer (GSP) that was chosen. An aliquot of the double stranded cDNA was added to the following reagents: 10 µM Marathon™ cDNA adapter, 5X DNA ligation buffer, T4 DNA ligase. The reaction was incubated at 16° C overnight and heat inactivated to terminate the reaction. PCR was performed by the addition of the following to the diluted double stranded cDNA pool: 10X cDNA PCR reaction buffer, 10

μM DNTP mix, 10 μM GSP, 10 μM AP1 primer (kit), 50X Advantage® cDNA Polymerase Mix. Thermal Cycling conditions were 94° C for 30 seconds, 5 cycles of 94° C for 5 seconds, 72° C for 4 minutes, 5 cycles of 94° C for 5 seconds, 70° C for 4 minutes, 23 cycles of 94° C for 5 seconds, 68° C for 4 minutes. After the first round of PCR was performed using the GSP to extend to the end of the adapter to create the adapter primer binding site, exponential amplification of the specific cDNA of interest was performed. Usually, a second, nested PCR was performed to provide specificity. The RACE product was analyzed on an agarose gel. Following excision from the gel and purification (GeneClean®, BIO 101), the RACE product was then cloned into pCTNR (General Contractor DNA Cloning System, 5'-3', Inc.) and sequenced to verify that the clone was specific to the gene of interest.

2. Expression Analysis. To characterize the expression of genes mapping to the 20p13-p12 region, a series of experiments were performed. First, oligonucleotide primers were designed for use in the polymerase chain reaction (PCR) so that portions of a cDNA, EST, or genomic DNA could be amplified from a pool of DNA molecules or RNA population (RT-PCR). The PCR primers were used in a reaction containing genomic DNA to verify that they generated a product of the predicted size (based on the genomic sequence). A critical piece of data that is required when characterizing novel genes is the length, in nucleotides, of the processed transcript or messenger RNA (mRNA). Those skilled in the art primarily determine the length of an mRNA by Northern analysis (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y. (1989)). Probes were generated using one of the methods described below. Briefly, sequence verified IMAGE consortium cDNA clones were digested with appropriate restriction endonucleases to release the insert. The restriction digest was electrophoresed on an agarose gel and the bands containing the insert were excised. The gel piece containing the DNA insert was placed in a Spin-X® (Corning Costar Corporation, Cambridge, Mass. or Supelco spin column (Supelco Park, Pa.) and spun at high speed for 15 mins. The DNA was ethanol precipitated and resuspended in TE. Alternatively, PCR products obtained from genomic DNA or RT-PCR were also purified as described above. Inserts purified from IMAGE clones were random primer labelled (Feinberg and Vogelstein) to generate probes for hybridization. Probes from purified PCR products were generated by incorporation of a- ^{32}P-dCTP in second round of PCR. Commercially available Multiple Tissue Northern blots (Clontech, Palo Alto, Calif.) were hybridized and washed under conditions recommended by the manufacturer. FIG. 16 depicts the Northern Analysis of Gene 216. As shown in the figure, various tissue sources showed expression of Gene 216.

3. RT-PCR. RT-PCR was used as an alternate method to Northern blotting to detect mRNAs with low levels of expression. Total RNA from multiple human tissues was purchased from Clontech (Palo Alto, Calif.) and genomic DNA was removed from the total RNA by DNaseI digestion. The "Superscript™ Preamplification System for First strand cDNA synthesis" (Life Technologies, Gaithersburg, Md.) was used according to manufacturer's specifications with oligo(dT) or random hexamers to synthesize cDNA from the DNaseI treated total RNA. Gene specific primers were used to amplify the target cDNAs in a 30 μl PCR reaction containing 0.5 μl of first strand cDNA, 1 μl sense

primer (10 µM), 1 µl antisense primer (10 µM), 3 µl dNTPs (2 mM), 1.2 µl $MgCl_2$ (25 mM), 3 µl 10X PCR buffer and 1 unit of Taq Polymerase (Perkin Elmer). The PCR reaction was initially denatured at 94° C for 4 min, then 30 cycles of denaturation at 94° C for 30 sec, annealing at 58° C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72° C for 7 min. PCR products were analyzed on agarose gels.

Please amend the specification at page 67, lines 10-29 as follows:

Multiple protein alignment of 19 Human a disintegrin-like and metalloproteinase-containing protein (ADAMs) and Gene 216 was performed using the GCG® program PILEUP (Wisconsin Package Version 9.1 Genetics Computer Group (GCG), 1997). The alignment was based on the full amino acid sequence of the 19 ADAMs and Gene 216, and utilized a gap creation penalty of 12 and a gap extension penalty of 4. The results of the alignment generated two outputs: a phylogenetic tree known as a dendogram that shows relatedness and evolutionary diversity of the genes to each other (FIG. 10); and an amino acid sequence alignment of those genes (FIGS. 11A-11D).

Multiple protein alignment of the predicted mouse homolog of Gene 216 and the alternately spliced variants, Gene 216a, Gene 216b and Gene 216c (FIGS. 12A-12B) was performed in GeneWorks® version 2.3 (IntelliGenetics). The alignment was based on the full amino acid sequence of the predicted mouse gene and Gene 216, and utilized a gap creation penalty of 12 and a gap extension penalty of 4.

The Kyte-Doolittle hydrophobicity plot (FIG. 13) was utilized in GeneWorks version® 2.3 (IntelliGenetics). This algorithm measures the hydrophobicity across a protein, thus providing an indication of the probable location of regions of Gene 216 that may interact with the lipid bilayer of the cell membrane. The black bar with the letter "A" indicates the signal peptide sequence. The transmembrane domain is located by the black bar with the letter "B."

Please amend the specification at page 69, lines 18-27 as follows:

The dendogram (FIG. 10) demonstrated that Gene 216 was probably most closely related to ADAM 12 and 19. The dendogram also indicated that 4 additional ADAMs were probably distantly related to Gene 216. Amino acid sequence alignment of these 6 ADAMs to Gene 216 (FIGS. 11A-11D) indicated regions of significant similarity that represented the domains which genes of this type possess. This alignment was determined by GCG® (Wisconsin Package Version 9.1 Genetics Computer Group (GCG), 1997). The alignment was based on a gap creation penalty of 12 and gap extension penalty of 4. Arrows represent the likely position of the domains, boxed amino acid residues represent the consensus regions in Gene 216 with ADAMs and dashed boxed amino acids represents a putative SH_3 binding site.

Please amend the specification at page 70, line 17-23 as follows:

All ADAMs also encode a highly conserved metalloprotease domain similar to the sequence (TMAHEIGHSLGLSHDPD; SEQ ID NO.:46) in Gene 216 (Table 7 and FIG. 11B). The 3 histidines (H) bind a zinc ion, the second glycine (G) allows a turn and the glutamic acid (E) is the catalytically active residue. This sequence is followed by a "Met turn", a structure that folds back and stabilizes the interaction with zinc. The presence of the metalloprotease domain and the "Met turn" suggests that Gene 216 has proteolytic activity.

Please amend the specification at page 78, line 26 through page 80, line 9 as follows (Please note that "ASO Assay" is not being added, rather it is underlined in the specification as filed):

2. ASO Assay. The amplicon, containing the polymorphism, was PCR amplified using primers that were used to generate a fragment for sequencing (sequencing primers) or SSCP (SSCP primers). The appropriate population of individuals was PCR amplified in 96 well microtitre plates and re-arrayed into 384 well microtitre plates using a Tecan Genesis® RSP200. The amplified products were loaded onto 2% agarose gels and size fractionated at 150V for 5 minutes. The DNA was transferred from the gel to Hybond N+ nylon membrane™ (Amersham-Pharmacia) using a Vacuum blotter (Bio-Rad). The filter containing the blotted PCR products was transferred to a dish containing 300 mls of pre-hybridization solution (5X SSPE {pH7.4}, 2% SDS, 5X Denhardts). The filter was left in the pre-hybridization solution at 40° C for >1 hour. After pre-hybridization, 10 mls of the pre-hybridization solution and the filter were transferred to a washed glass bottle. The allele specific oligonucleotides (ASO) were designed with the polymorphism in the middle. The size of the oligonucleotide was dependent upon the GC content of the sequence around the polymorphism. Those ASOs that had a G or C polymorphism were designed so that the Tm was between 54-56° C and those that had an A or T variance were designed so that the Tm was between 60-64° C All oligonucleotides were phosphate free at the 5'end and purchased from Gibco BRL. For each polymorphism 2 ASOs were designed: one for each variant.

The two ASOs that represented the polymorphism were resuspended at a concentration of 1 µg/µl and separately end-labeled with γ-ATP^{32} (6000 Ci/mmol) (NEN) using T4 polynucleotide kinase according to manufacturer recommendations (NEB). The end-labeled products were removed from the unincorporated γ-ATP^{32} by passing the reactions through Sephadex® G-25 columns (*i.e.*, cross-linked dextran beads) according to manufacturers recommendation (Amersham-Pharmacia). The entire end-labeled product of one ASO was added to the bottle containing the appropriate filter and 10 mls of hybridization solution. The hybridization reaction was placed in a rotisserie oven (Hybaid) and left at 40° C for a minimum of 4 hours. The other ASO was stored at -20° C.

After the prerequisite hybridization time had elapsed, the filter was removed from the bottle and transferred to 1 liter of wash solution (0.1X SSPE {pH7.4}, 0.1% SDS) pre-warmed to 45° C. After 15 minutes the filter was transferred to another liter of wash solution (0.1X SSPE {pH7.4}, 0.1% SDS) pre-warmed to 50° C. After 15 minutes the filter was wrapped in Saran®, placed in an autoradiograph cassette and an X-ray film (Kodak) placed on top of the filter. Depending on the efficiency of the end-labeling reaction of the ASO and its hybridization to the filter an image would be observed on the film within an hour. After an image had been captured on film for the 50° C wash, the process was repeated for wash steps at 55° C, 60° C and 65° C. The image that captured the best result was used.

Please amend the specification at page 80, lines 21-24 as follows:

The two films that best captured the allele specific assay with the two ASOs were converted into digital images by scanning them into Adobe PhotoShop®. These images were overlaid against each other in Graphic Converter and then scored and stored in FileMaker® Pro 4.0.

Please amend the specification at page 92, lines 14-30 as follows:

DNA prepared from the 20p13-p12 region is used as the source of template DNA for PCR amplification (Ausubel et al, *Current Protocols in Molecular Biology*, John Wilty & Sons (1994)). To amplify a DNA sequence containing the nucleotide sequence, c DNA (50 ng) is introduced into a reaction vial containing 2 mM $MgCl_2$, 1 micromolar synthetic oligonucleotide primers (forward and reverse primers) complementary to and flanking a defined 20p13-p12 region, 0.2 mM of each of deoxynucleotide triphosphate, dATP, dGTP, dCTP, dTTP and 2.5 units of heat stable DNA polymerase (Amplitaq®, Roche Molecular Systems, Inc., Branchburg, N.J.) in a final volume of 100 microliters.

Upon completion of thermal cycling reactions, each sample of amplified DNA is purified using the Qiaquick® Spin PCR purification kit (Qiagen, Gaithersburg, Md.). All amplified DNA samples are subjected to digestion with the restriction endonucleases, e.g., NcoI and EcoRI (New England BioLabs, Beverly, Mass., U.S.A.) (Ausubel et al, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994)). DNA samples are then subjected to electrophoresis on 1.0% NuSeive (FMC BioProducts, Rockland, Me.) agarose gels. DNA is visualized by exposure to ethidium bromide and long wave UV irradiation. DNA contained in slices isolated from the agarose gel are purified using the Bio 101 GeneClean® Kit protocol (Bio 101, Vista, Calif.).

Please amend the specification at page 94, lines 5-13 as follows:

The pET vector can be propagated in any *E. coli* K-12 strain, e.g., HMS174, HB101, JM109, DH5® competent cells and the like, for purposes of cloning or plasmid preparation. Hosts for expression include E. coli strains containing a chromosomal copy of the gene for T7 RNA polymerase. These hosts are lysogens of bacteriophage DE3, a lambda derivative that carries the lacI gene, the lacUV5 promoter and the gene for T7 RNA polymerase. T7 RNA polymerase is induced by addition of isopropyl-.beta.-D-thiogalactosi- de (IPTG), and the T7 RNA polymerase transcribes any target plasmid containing a functional T7 promoter, such as pET-28b, carrying its gene of interest. Strains include, for example, BL21(DE3) (Studier *et al, Meth. Enzymol.*, 185:60-89 (1990)).

Please amend the specification at page 41-42, Table 2 to include the "SEQ ID NO." column, as follows:

Table 2:

Overgo	Locus	DNA Type	Gene	Forward Primer	SEQ ID NO.	Reverse Primer	SEQ ID NO.
stSG24277		Genomic		AACTCTTGAAATGAGAAGCGTG	8	CGGATTCACGCTTC	183
stSG408		EST		AATATCATGCACCATGACCCAC	9	ATGGCTGTGGGTCA	184
A005O05		EST	Attractin (ATTN)	TGGAGTAAGTATTGTAAACTAT	10	AATGAAATAGTTTA	185
B849D17AL		BACend		GGAGCTTATCCTGGATTATCTA	11	CCCACTTAGATAAT	186
SN2		EST	Sialoadhesin (SN)	AGAGCCACACATCCATGTCCTG	12	GGGAAGCCAGGACAT	187
AFMb026xh5	D20S867	MSAT		AAGCCACTCTGTGAATTGCCAT	13	GAGGCAATGGCAAT	188
SN1		EST	Sialoadhesin (SN)	GAGTAGTCGTAGTACCAGATGG	14	ATCACGGCCATCTGG	189
stsH22126		EST		GTCTGGCAATGGAGCATGAAAA	15	TCATTCATTTTCATG	190
W14876	D20S752	Genomic		ATTAGAGCACATGAAGGAAAGG	16	ACTTCTCCTTTCCT	191
stSG30448		EST		ACACTGCTTTGGGGGACAGGCT	17	AGACCTAGCCTGTC	192
W118677		EST		CACGACGCCACAGAGCCAGCTC	18	GGACGGAGCTGGC	193

Please amend the specification at page 56-57 to include the "SEQ ID NO." column, Table 3 as follows:

TABLE 3: Sequence and tissue distribution of the paired linkers

Paired linkers	Sequence	SEQ ID No.	Cell/Tissue Type
OLIGO 3	5'CTC GAG AAT TCT GGA TCC TC3'	34	Th2/unstimulated(dT+rp)
OLIGO 4	5'TTG AGG ATC GAG AAT TCT CGA G3'	35	Th0/stimulated/anti CD3 (dT+rp) Pulmonary artery endothelium cells (dT+rp) Lung microvascular endothelial cells (dT+rp) Bronchial epithelium cells (dT+rp)
OLIGO 5	5'TGT ATG CGA ATT CGC TGC GCG3'	36	Normal Lung (dT+rp)
OLIGO 6	5'TTC GCG CAG CGA ATT CGC ATA CA3'	37	Athmatic lung (dT+rp) Th2/stimulated/TPA (dT+rp) Bronchial smooth muscle cells (dT+rp)
OLIGO 9	5'CCT ACG GAA TTC TCA CTC AGC3'	38	Brain (dT+rp)
OLIGO 10	5'TTG CTG AGT GAG AAT TCC GTA GG3'	39	Th0/unstimulated (dT+rp) Pulmonary artery smooth muscle cells (dT+rp)
OLIGO 11	5'GAA TCC GAA TTC CTG GTC AGC3'	40	Lung fibroblasts (dT+rp)
OLIGO 12	5'TTG CTG ACC AGG AAT TCG GAT TC3'	41	Th0/stimulated/TPA (dT+rp) Small airway epithelium cells (dT+rp)

Please amend the specification at page 58-59, Table 4 to include the "SEQ ID NO." column, as follows:

TABLE 4: Sequence of the 5 modified oligonucleotides used to amplify the secondary selected material prior to cloning into the pAMP10 vector.

Modified Oligonucleotides	Sequence	SEQ ID No.
OLIGO 3	5'CUA CUA CUA CUA CTC GAG AAT TCT GGA TCC TC3'	42
OLIGO 5	5'CUA CUA CUA CUA TGT ATG CGA ATT CGC TGC GCG3'	43
OLIGO 9	5'CUA CUA CUA CUA CCT ACG GAA TTC TCA CTC AGC3'	44
OLIGO 11	5'CUA CUA CUA CUA GAA TCC GAA TTC CTG GTC AGC3'	45

Please amend the specification at page 70, Table 7 to include the "SEQ ID NO." column, as follows:

TABLE 7: shows the top two hits when Gene 216 was compared against the motif database using Blimps. The disintegrin and metalloproteinase domains were identified.

Description	Strength	Score	AA#	AA Sequence	SEQ ID No.
Disintegrins proteins	1950	1597	377	CCfAhnCsLRPGAQCAh GdCCvRC11KpAGal CRqAMGDCD1PEfCT GTSshCPP	47
Zinc metallopeptidases	1173	1276	276	TMAHEIGHSLG	48

Please amend the specification at page 74-75, Table 8 to include the "SEQ ID NO." column, as follows:

TABLE 8:

Gene	Exon	Assay Name	PrimerSequence	SEQ ID No.	PrimerSequence	SEQ ID No.
216	216_A	291_216_A_F_292_216_A_R	TCACAGCTATGGGCTGGAG	49	GAGCTCTGAGCAGAACCCAT	76
216	216_A	502_216_A_F_503_216_A_R	CTGCCTAGAGGCCGAGGA	50	AGCTCTGAGCAGAACCCATC	77
216	216_B	293_216_B_F_294_216_B_R	CCCCTGTGTTCCTCAGGTC	51	AGTGACTTGGTGGTTCTGGG	78
216	216_C	295_216_C_F_296_216_C_R	GCTCCACACTCTTTCTTGCC	52	TGTCATCTGCACCCTCTCTG	79
216	216_D	297_216_D_F_298_216_D_R	AGGCAGGAGGAAGCTGAAT	53	AAGAGGGAGGGTGTGGTAGG	80
216	216_F	299_216_F_F_300_216_F_R	CCTACCCCTCTGCACCCTA	54	ATACAGCATTCCCACTCCCA	81
216	216_G	301_216_G_F_302_216_G_R	AACTTCCTTCTGGGAGCTGG	55	GAAGGCAGAAATCCCGGT	82
216	216_H	303_216_H_F_304_216_H_R	CAAGCCACCGGGATTTCT	56	CCCTTCCTCTTCCCCAAAC	83
216	216_H	700_216_H_F_701_216_H_R	CACACCCTGGTGAGGAGAGA	57	CACCAGCACCTGCCTGTC	84
216	216_I	305_216_I_F_306_216_I_R	CCACGAAGGACCACCG	58	GGGTCAGAGGCACCCAC	85
216	216_J	307_216_J_F_308_216_J_R	GTGGGTGCCTCTGACCC	59	AGAGCCTCCTGTCTCTCCCT	86
216	216_J	703_216_J_F_734_216_J_R	CACGTGGGTGCCTCTGAC	60	GGGTCAGAGGCACCCAC	87
216	216_J	889_216_J_F_890_216_J_R	CTCACGTGGGTGCCTCTG	61	GCCGTAGAGCCTCCTGTCT	88
216	216_K	309_216_K_F_310_216_K_R	AGAGACAGGAGGCTCTACGG	62	AAGTCCCCAGGACTAGCCG	89
216	216_K	309_216_K_F_704_216_K_R	AGAGACAGGAGGCTCTACGG	63	GAAACTGAGGGACGACCAAA	90
216	216_K	891_216_K_F_892_216_K_R	CTCTACGGCCGCAGTGAC	64	GACGACCAAAGAAACGCAG	91
216	216_L	311_216_L_F_312_216_L_R	GTCCCTCCATGCCCAATG	65	TGAGCGGAGAGGGCAAGT	92
216	216_L	313_216_L_F_314_216_L_R	CAGGTTAAGTCGGCTCGC	66	AAACCCTCACCCTGAACCTT	93
216	216_M	315_216_M_F_316_216_M_R	CTCTGTCTGCCTTCCCCAC	67	AAGGGTGCTCGTGTCGTCT	94
216	216_N	317_216_N_F_318_216_N_R	TCTACTGTGGGGAAGATGGG	68	CCACTCAGCTCCACTCCCTA	95
216	216_O	319_216_O_F_320_216_O_R	CCCCTCTACTTCCTCCCCA	69	GGATTCAAACGGCAAGGAG	96
216	216_P	321_216_P_F_322_216_P_R	GACCTTGGGGTTCCTAATCC	70	GCTGAGTCCTGAGCAGGTG	97
216	216_Q	323_216_Q_F_324_216_Q_R	GTGCACCTGCTCAGGACTC	71	GCAGGAGTAGGCTCAGGAAG	98
216	216_Q	323_216_Q_F_504_216_Q_R	GTGCACCTGCTCAGGACTC	72	GAACCGCAGGAGTAGGCTC	99
216	216_R	325_216_R_F_326_216_R_R	CCTGGACTCTTATCACGTTGC	73	ATATGGTCAGCAGGAGACCC	100
216	216_S	327_216_S_F_328_216_S_R	TTACCCTCCACCATTTCTCC	74	GCATCCTGGTCTCCATGATAA	101
216	216_T	985_216_T_F_986_216_T_R	TTCCTGGATCACTGGTCCTC	75	CGGTGATTCACTGGCTCTG	102

Please amend the specification at page 75, Table 9 to include the "SEQ ID NO." column, as follows:

TABLE 9:

Exon	Forward	ForwardSeq	SEQ ID No.	ReverseName	ReverseSeq	SEQ ID No.
216_A	MDSeq_101_216_A_F	CCTCTCAGGAGTAGAGGCCC	103	MDSeq_101_216_A_R	CCAAGCACACTTGAGCGTC	119
216_A	MDSeq_175_216_A_F	AGCGGTTCTCTCCTCCTCTC	104	MDSeq_175_216_A_R	AGCCATGCCCTCTGCTTT	120
216_A	MDSeq_79_216_A_F	GCACGGATTCCCTCCTCC	105	MDSeq_79_216_A_R	AGCCATGCCCTCTGCTTT	121
216_D	MDSeq_61_216_D_F	TCCCTGGTGCTTCCCATA	106	MDSeq_61_216_D_R	GAGGGAGCTCTTTCCCCA	122
216_F	MDSeq_47_216_F_F	CCACTACCAAGOGCGAGTAA	107	MDSeq_47_216_F_R	AGTTCCAGGTACTTTCCGGGT	123
216_F	MDSeq_57_216_F_F	CCTCTTGCCCCTCTTGCT	108	MDSeq_57_216_F_R	AACCCCAGGTCCCAGAAG	124
216_H	MDSeq_155_216_H_F	GGCCTCGAGTCCCAGTATTT	109	MDSeq_155_216_H_R	ACTGCAGGAAGGCCCAGAG	125
216_J	MDSeq_181_216_J_F	TCGCCCTCAGCTTCTCAG	110	MDSeq_181_216_J_R	TGAGGGACGACCAAAGAAAC	126
216_K	MDSeq_182_216_K_F	TCACGTGGGTGCCTCTGA	111	MDSeq_182_216_K_R	CAAAGTCACACAACAAGCGG	127
216_L	MDSeq_106_216_L_F	GGGTTACTTCCCCTCTCTGG	112	MDSeq_106_216_L_R	GAACCTGAGOGCACCAATTTA	128
216_L	MDSeq_48_216_L_F	CCTGTCCCGCTTGTTGTGT	113	MDSeq_48_216_L_R	ACGTGCAGTGAGAGGTCCAT	129
216_L	MDSeq_56_216_L_F	CGGGCTGCTCACTATTGG	114	MDSeq_56_216_L_R	GAGAGGTCCATGCCGAGA	130
216_L	MDSeq_67_216_L_F	GCGAGGUACTCCTACACCG	115	MDSeq_67_216_L_R	AAGGTTCAGGGTGAGGGTTT	131
216_O	MDSeq_49_216_O_F	TCCAGGTGGTGAACTCTGC	116	MDSeq_49_216_O_R	CTGGAGCACAGTGGCAGTTA	132
216_Q	MDSeq_96_216_Q_F	GACCTTGGGGTTCCTAATCC	117	MDSeq_96_216_Q_R	TGTACTGGGAGGTAGAGGGC	133
216_R	MDSeq_50_216_R_F	AGAGGGTGACTTGGAGCAGA	118	MDSeq_50_216_R_R	CCAGAAACCTGATTAGGGGG	134

Please amend the specification at page 77, Table 10 to include the "SEQ ID NO." column and to add the SNP sequences, as follows:

TABLE 10:

D	GTGCTTCCCATATTCACATCTCCCACAACTAAGCCATCAC GTGCTTCCCATATTCACACCTCCCACAACTAAGCCATCAC	SEQ ID NO.: 135 SEQ ID NO.: 136	T>C	Intron	7521
D	AACTAAGCCATCACCAAGGCTCCTTCCTCTAGCCCCAAG AACTAAGCCATCACCAAGCCTCCTTCCTCTAGCCCCAAG	SEQ ID NO.: 137 SEQ ID NO.: 138	G>C	Intron	7547
D	GGATACATAGAAACCCACTACGGCCCAGATGGGCAGCCA GGATACATAGAAACCCACCACGGCCCAGATGGGCAGCCA	SEQ ID NO.: 139 SEQ ID NO.: 140	T>C	Exon	7772
F	CTGCTCACCTGGAAAGGAACCTGTGGCCACAGGGATCCT CTGCTCACCTGGAAAGGAGCCTGTGGCCACAGGGATCCT	SEQ ID NO.: 141 SEQ ID NO.: 142	A>G	Exon	8271
F	CTCCAAATCAGAAGAGACAGGAATTCACAGGCCTCGAGT CTCCAAATCAGAAGAGACGGGAATTCACAGGCCTCGAGT	SEQ ID NO.: 143 SEQ ID NO.: 144	A>G	Intron	8405
I	CCTGCAGTGGCGCCGGGGGCTGTGGGCGCAGCGGCCCCA CCTGCAGTGGCGCCGGGGACTGTGGGCGCAGCGGCCCCA	SEQ ID NO.: 145 SEQ ID NO.: 146	G>A	Intron	9057
L	CCCTCTCTGGGCTCTGCGCGTCTGGCGGCTGTAGCCAAG CCCTCTCTGGGCTCTGCGCATCTGGCGGCTGTAGCCAAG	SEQ ID NO.: 147 SEQ ID NO.: 148	G>A	Intron	9848
L	GAGAAGCGCGGGGGTTGGGGGACTGTCCCTCCATGCCCA GAGAAGCGCGGGGGTTGGAGGACTGTCCCTCCATGCCCA	SEQ ID NO.: 149 SEQ ID NO.: 150	G>A	Intron	9903
L	AGCCGCCGCCCAGCTGCGCGCCTTCTTCCGCAAGGGGGGC AGCCGCCGCCAGCTGCGCGTCTTCTTCCGCAAGGGGGGC	SEQ ID NO.: 151 SEQ ID NO.: 152	C>T	Exon	9995
L	GTTCAGGGTGAGGGTTTCGGGGAGCTTGGGAGCCGGCCT GTTCAGGGTGAGGGTTTCGTGGAGCTTGGGAGCCGGCCT	SEQ ID NO.: 153 SEQ ID NO.: 154	G>T	Intron	10341
O	TGAGCTCTGCCCACCCGACCCCTCCTTGCCGTTTGAATCC TGAGCTCTGCCCACCCGACTCCTCCTTGCCGTTTGAATCC	SEQ ID NO.: 155 SEQ ID NO.: 156	C>T	Intron	11283
Q	GCTGGCCATGCTCCTCAGCGTCCTGCTGCCTCTGCTCCCA GCTGGCCATGCTCCTCAGCATCCTGCTGCCTCTGCTCCCA	SEQ ID NO.: 157 SEQ ID NO.: 158	G>A	Exon	11725
Q	TCCTGCTGCCTCTGCTCCCAGGGGCCGGCCTGGCCTGGTG TCCTGCTGCCTCTGCTCCCAGGCGCCGGCCTGGCCTGGTG	SEQ ID NO.: 159 SEQ ID NO.: 160	G>C	Exon	11748
Q	GTGGCCTCCCAGTCAAGCGAGGGGGTGGATCCCTGCCCC GTGGCCTCCCAGTCAAGCGTGGGGGTGGATCCCTGCCCC	SEQ ID NO.: 161 SEQ ID NO.: 162	A>T	Intron	12018
R	CTGGGCGGCGTTCACCCCATGGAGTTGGGCCCCACAGCC CTGGGCGGCGTTCACCCCACGGAGTTGGGCCCCACAGCC	SEQ ID NO.: 163 SEQ ID NO.: 164	T>C	Exon	13263
R	AGTTGGGCCCCACAGCCACTGGACAGCCCTGGCCCCTGG AGTTGGGCCCCACAGCCACTGGACAGTCCTGGCCCCTGG	SEQ ID NO.: 165 SEQ ID NO.: 166	C>T	Exon	13292
R	GGGCTCATGCCTCCTGCCTCCTTCCAGATGGGCAGCACCC GGGCTCATGCCTCCTGCCTTCTTCCAGATGGGCAGCACCC	SEQ ID NO.: 167 SEQ ID NO.: 168	C>T	Intron	13370
R	TATGCCCCTCCCCAGCCCAGGGTCTCCTGCTGACCATAT TATGCCCCTCCCCAGCCCAGGGGCTCCTGCTGACCATAT	SEQ ID NO.: 169 SEQ ID NO.: 170	T>G	Intron	13431

Please amend the specification at page 85-86, Table 12 to include the "SEQ ID NO." column, as follows:

TABLE 12:

U.S. and UK Samples

	Control	Case	P-value	SEQ ID No.
Pro-Met-Ile-Ser-Tyr	74.7%	79.2%	0.1692	171
Ser-Thr-Ile-Ser-Tyr	10.5%	11.7%	0.6598	172
Pro-Met-Val-Ser-Tyr	10.0%	5.0%	0.0274	173
Pro-Met-Ile-Asn-Tyr	3.2%	3.1%	0.9850	174
Pro-Thr-Val-Ser-Tyr	0.8%	0.0%	0.3589	175
Ser-Met-Ile-Ser-Tyr	0.6%	0.4%	0.6641	176
Ser-Met-Val-Ser-Tyr	0.2%	0.0%	0.8713	177
Pro-Met-Ile-Ser-His	0.0%	0.4%	0.2210	178
Ser-Thr-Val-Ser-Tyr	0.0%	0.1%	0.0397	179
Pro-Thr-Ile-Ser-Tyr	0.0%	0.0%	0.7012	180
Overall			0.2244	

UK Samples

	Control	Case	P-value	SEQ ID No.
Pro-Met-Ile-Ser-Tyr	74.6%	84.4%	0.0120	171
Ser-Thr-Ile-Ser-Tyr	12.1%	8.3%	0.1901	172
Pro-Met-Val-Ser-Tyr	9.7%	4.9%	0.0604	173
Pro-Met-Ile-Asn-Tyr	2.0%	1.6%	0.7945	174
Pro-Thr-Val-Ser-Tyr	1.1%	0.0%	0.3842	175
Ser-Met-Val-Ser-Tyr	0.4%	0.0%	0.7954	177
Pro-Met-Val-Asn-Tyr	0.2%	0.0%	0.7767	181
Pro-Met-Ile-Ser-His	0.0%	0.5%	0.1826	178
Ser-Thr-Ile-Asn-Tyr	0.0%	0.3%	0.0868	182
Ser-Thr-Val-Ser-Tyr	0.0%	0.1%	0.0568	179
Pro-Thr-Ile-Ser-Tyr	0.0%	0.0%	0.5109	180
Overall			0.0930	

US Samples

	Control	Case	P-value	SEQ ID No.
Pro-Met-Ile-Ser-Tyr	75.2%	60.6%	0.0384	171
Pro-Met-Val-Ser-Tyr	10.4%	6.1%	0.4397	173
Ser-Thr-Ile-Ser-Tyr	7.8%	24.1%	0.0040	172
Pro-Met-Ile-Asn-Tyr	5.2%	7.4%	0.5083	174
Ser-Met-Ile-Ser-Tyr	1.5%	1.9%	0.9707	176
Ser-Thr-Val-Ser-Tyr	0.0%	0.0%	0.5606	179
Overall			0.0659	